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(ii) fertilizing the reconstructed oocyte to produce a reconstructed zygote;

(ii) transferring the reconstructed zygote into an oviduct of a recipient female of the same species as the zygote; and

(iii) allowing the reconstructed zygote to develop to term.

Please cancel claim 31.

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32. A method of preparing a recipient cell comprising the steps of:

(i) providing a cell having a nucleus therein;

(ii) visualizing the nucleus using light in the near-infrared region; and

B7 (iii) ablating the nucleus to provide an enucleated recipient cell.

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33. The method of claim 32 wherein the nucleus is visualized and ablated via two photon laser scanning microscopy.

REMARKS

Claims 1-6, 11, 14-22 and 24-33 are pending in the application. Claims 1-6, 11, 14-22 and 24-33 are rejected. Claims 1, 14, 19, 21, 24-26, 28 and 30 have been amended. Claims 11, 27, 29 and 31 have been canceled. Entry of the amendment is respectfully requested.

The Amendment

Claims 1, 14, 19, 21, 24-26, 28 and 30 have been amended. All amended claims are supported by the application as filed. No new matter was added by this amendment.

Claim 1 has been amended to specify that the claimed avian zygote or oocyte is a "chicken" zygote or oocyte "for generation of a transgenic chicken". Support for this amendment can be found on page 10, lines 11-14; page 11, lines 4-5, line 9, and line 20; and page 13, lines 1-2 of the specification. The claim has been further amended to add a step of "activating the reconstructed zygote or oocyte" and "allowing the reconstructed

zygote or oocyte to develop to term". Support for this amendment can be found on page 10, lines 12-13 of the specification.

Claim 14 has been amended to specify that the claimed avian is a "chicken". Support for this amendment can be found on page 18, lines 7-11. A further amendment specifies that the avian zygote and oocyte is a "chicken" zygote or oocyte. Support for this amendment can be found on page 10, line 14 and lines 16-19 of the specification.

Claim 19 has been amended to specify that the claimed transgenic avian is a "chicken". A further amendment specifies that the avian recipient cell, avian oocytes, avian donor nucleus, and avian zygote or oocyte are "a chicken" recipient cell, "chicken" oocytes, "chicken" donor nucleus, and "chicken" zygote or oocyte, respectively. Support for this amendment can be found on page 10, line 14; page 12, lines 1-8; and page 28, lines 1-6 of the specification.

Claim 20 has been amended to add monoclonal and polyclonal antibodies. Support for this amendment can be found on page 28, line 13 (see WO99/10505).

Claim 21 has been amended to specify that the transgenic avian is a transgenic "chicken". Additional minor amendments clarify that the protein is "being" deposited "into" the developing "chicken eggs" and that the harvested hard shell eggs are those of a "chicken". Support for this amendment can be found on page 10, line 14; page 28, lines 1-6; and page 30, lines 11-15 of the specification.

Claim 24 has been amended to depend on claim 14; and has further been amended to specify that the "cloned chicken" is a knock-out or knock-in "chicken". Support for this amendment can be found on page 28, lines 19-20 and on page 29, lines 1-3 of the specification.

Claim 25 has been amended to indicate that the claim is dependent on "claim 21" and that the hard shell egg contains "exogenous protein". Support for this amendment can be found on page 30, lines 15-16.

Claim 26 has been amended to specify that the "reconstituted avian" embryo is a "reconstructed chicken" embryo that "comprises a nucleus from a first donor cell in the

cytoplast of a second suitable recipient cell". Support for this amendment can be found on page 13, lines 1-2 and on page 18, line 19 of the specification.

Claims 28 and 30 have both been amended to specify that the cloned avian is a cloned "chicken". Support for this amendment can be found on page 10, line 14. Claims 28 and 30 have also both been amended to replace the "process" of claim 1 with the "method" of claim 1 to provide for proper antecedent basis.

Rejections under 35 U.S.C. §112

Claims 1-6, 11, 14-22 and 24-33 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention.

The Examiner maintains that the state of the art of nuclear transfer is not predictable with regard to the production of cloned animals and cloned birds. The Examiner asserts that the specification does not provide sufficient teachings or guidance to show that cloned avian could be produced by the claimed method of nuclear transfer, as the specification only putatively describes how ova prepared by nuclear transfer could be transferred into recipient hens which could then lay eggs.

To the extend that the rejection applies to the claims as amended, Applicants respectfully traverse the rejection. In the interest of prosecution efficiency, Applicants have amended the claims to refer to chickens rather than avians (*supra*). Applicants refer the Examiner to the Example section (pages 31-36) wherein the invention is illustrated with chickens. Applicants have established through these specific working examples that their procedures result in a reconstructed chicken zygote or oocyte which ultimately develops into a cloned chicken. Specifically, Applicants have shown that ova were isolated from euthanized hens (*i.e.*, chickens) (page 31, line 16) or alternatively eggs were isolated from hens with fistulated oviducts (page 31, line 17); dye was injected into the germinal disk of the ovum (page 31, lines 11-12); images of the inside of the avian early embryo (*i.e.*, chicken embryo) were obtained through the use of TPLSM by placing

the germinal disk on the microscope and searching for the pronuclear structures within the central area of the disk (page 32, lines 15-18); once found, the nuclear structures were ablated through laser mediated ablation (page 32, line 19 and page 33, lines 1-10); a donor nucleus was isolated (page 34, line 3); and a reconstructed zygote was prepared via localization and positioning of the germinal disk under the microscope and subsequently guided injection of the somatic cells (page 34, lines 13-15). Furthermore, the donor ovum (page 35, line 4) is then placed into a recipient hen through a surgical procedure (page 35, line 8) wherein the ovum is allowed to move into the infindibulum and into the interior magnum by gravity feed (page 35, lines 13-14). The recovery time for the bird is specified as 45 minutes (page 25, line17). The eggs laid by the recipient hen are collected the next day and incubated; the chicks hatch 21 days later (page 35, lines 18-19). Applicants contend that one of ordinary skill in the art would find Applicants' disclosure enabling with respect to claims 1-6, 11, 14-22 and 24-33. In light of the amendment, the claims are now clearly in condition for allowance.

It is also emphasized (as in previous responses) that the specification teaches visualization of the nuclear material by using light in the near-infrared region (*i.e.*, two photon laser scanning microscopy (TPLSM)) for nuclear transfer in avians. The large size and optical density of the yolk have made the visualization of the avian early embryo and its structures difficult in the past (see page 6, lines 11-20 of the specification). Using light in the near-infrared region is a new method with which Applicants have accomplished the visualization of the early avian egg which is a paramount achievement in the art of avian nuclear transfer. Through this new method the skilled artisan will now be able to visualize the target structures in the early avian embryo with relative ease. Applicants describe on page 32-33 of the specification how TPLSM was used to visualize the avian early embryo and to subsequently ablate nuclear structures. Although, this process is applicable to all avian species, it is particularly applicable to chickens as described in the specification (*supra*).

The Examiner also contends that the method steps of the claims do not enable the claimed invention because the claims do not include an activation step. Applicants have

amended claim 1 to add a step of activating the reconstructed zygote or oocyte (*supra*) which is supported by the specification on page 10, lines 12-13 of the specification.

The Examiner further asserts that the specification does not provide specific teachings or guidance as to how to use the claimed reconstructed avian zygotes or oocytes, expect for the generation of cloned or transgenic avians. Applicants have amended claim 1 to indicate that the method is used "for generation of a transgenic chicken" (*supra*) which is supported on page 10, lines 11-14 and page 11, lines 4-5 and line 16.

In light of the foregoing amendment and remarks, Applicants respectfully request withdrawal of the rejection of claims 1-6, 11, 14-22, 24-26 and 28-33 under 35 U.S.C. §112, first paragraph.

Claims 25 and 27-31 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter.

The rejection is respectfully traversed.

The Examiner contends that the metes and bounds of the term "normal complement" in claim 25 that recites that the hard-shell egg contains "less than the normal complement" of endogenous proteins found in the egg, are not defined by the claim. Applicants have amended claim 25 to indicate that the claim is dependent on "claim 21" and that the hard shell egg contains "exogenous protein". Support for this amendment can be found on page 30, lines 15-16.

The Examiner states that claim 27 is unclear because it recites "an embryo" and it is suggested that the claim be written to state "the embryo". Claim 27 has been canceled.

The Examiner states that there is insufficient antecedent basis for "the process of claim 1" as recited in claims 28 and 30. Applicants have amended the claims accordingly. Both, claims 28 and 30 have been amended to replace the "process" of claim 1 with the "method" of claim 1 to provide for proper antecedent basis. Dependent claims 29 and 31 have been canceled.

In light of the foregoing amendment and remarks, Applicants respectfully request withdrawal of the rejection of claims 25, 28 and 30 under 35 U.S.C. §112, second paragraph.

Rejections under 35 U.S.C. §102

Claims 26 and 27 are rejected under 35 U.S.C. §102(b), as allegedly being anticipated by Chang *et al.* (*Cell Biology International* (1997) 21:495-499).

The rejection is respectfully traversed.

The Examiner states that claim 26 is directed to a reconstituted avian embryo prepared by transferring the nucleus of a donor cell into a suitable recipient cell and that claim 27 is directed to the embryo of claim 26 in which the donor cell is quiescent. The Examiner further states that the claims are product by process claims and that the patentability of a product does not depend on its method of production. The Examiner also contends that if the product in the product-by process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process. The Examiner concludes that since Chang *et al.* teach the injection of cultured PGCs into recipient embryos obtained from the Korean native ogol chicken, Chang *et al.* anticipate the claimed invention.

For clarification, Applicants have amended claim 26 to specify that the "reconstituted avian" embryo is a "reconstructed chicken" embryo and to define the embryo. Support for this amendment can be found on page 13, lines 1-2 and on page 18, line 19 of the specification. Claim 27 has been canceled. Applicants claim a "reconstructed embryo" which is a term that has a meaning in the art. Specifically, the art understands the definition of a "reconstructed embryo" to mean an embryo that results from the transfer of a donor nucleus (nuclear donor) into a cytoplast (recipient cell). Applicants describe a reconstructed embryo on page 18, lines 17-19, wherein they state that a reconstructed embryo is produced by preparation of a chromosome-free recipient cell called a cytoplast (which involves chromosome removal often referred to as enucleation); donor cell nucleus (nuclear donor) isolation; and transfer of the nuclear

donor to the cytoplast. Thus, there exists a consensus in the art of what a "reconstructed embryo" means, namely an embryo wherein the nucleus of a donor adult cell was inserted into the enucleated egg. In that, the reconstructed embryo is not merely defined by its process but by its inherent property.

In comparison, Chang *et al.* teach the injection of cultured primordial germ cells into the blood stream of recipient chicken embryos from Korean native ogol chicken (see page 496, column 1, third paragraph). However, Chang *et al.* do not teach a reconstructed chicken embryo that results from transferring the nucleus of a donor cell into a cytoplast. Thus, Chang *et al.* do not anticipate claim 26.

> Anticipation requires identity of invention. The claimed invention, as described in appropriately construed claims, must be the same as that of the reference in order to anticipate. *Glaverbel Societe Anonyme v. Northlake Marketing & Supply Inc.*, 45 F.3d 1550, 33 USPQ.2d 1496, 1498 (Fed. Cir. 1995).

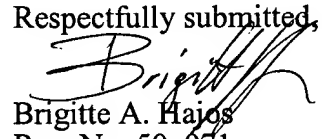
In light of the foregoing amendment and remarks, Applicants respectfully request withdrawal of the rejection of claim 26 under 35 U.S.C. §102(b).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claim 1 has been amended as follows:

1. (amended twice) A method of producing a reconstructed ~~avian~~ chicken zygote or oocyte for generation of a transgenic chicken, comprising the steps of:

(i) providing a recipient cell selected from the group consisting of ~~avian~~ chicken oocytes arrested at metaphase II and pronuclear zygotes;

(ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;

(iii) enucleating the recipient cell; ~~and~~

(iv) introducing a donor nucleus from the same species as the recipient cell into the recipient cell to produce the reconstructed ~~avian~~ chicken zygote or oocyte;

(v) activating the reconstructed zygote or oocyte; and

(vi) allowing the reconstructed zygote or oocyte to develop to term.

Claim 11 has been canceled.

Claim 14 has been amended as follows:

14. (amended twice) A method of producing a cloned ~~avian~~ chicken comprising the steps of:

(i) providing a recipient cell selected from the group consisting of ~~avian~~ chicken oocytes arrested at metaphase II and pronuclear zygotes;

(ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;

(iii) enucleating the recipient cell using light in the near infrared region;

(iv) introducing a donor nucleus from the same species as the recipient cell into the recipient cell to produce a reconstructed ~~avian~~ chicken zygote or oocyte;

(v) activating the reconstructed zygote or fertilizing the reconstructed oocyte;

(vi) transferring the reconstructed zygote or fertilized oocyte into an oviduct of a recipient female of the same species as the zygote or oocyte; and

(vii) allowing the reconstructed zygote or oocyte to develop to term.

Claim 19 has been amended as follows:

19. (amended twice) A method of producing a transgenic ~~avian~~ chicken comprising the steps of:

(i) providing ~~an avian~~ a chicken recipient cell selected from the group consisting of ~~avian~~ chicken oocytes arrested at metaphase II and pronuclear zygotes;

(ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;

(iii) enucleating the recipient cell;

(iv) introducing a transgenic ~~avian~~ chicken donor nucleus from the same species as the recipient cell into the recipient cell to produce a reconstructed ~~avian~~ chicken zygote or oocyte;

(v) activating the reconstructed zygote or fertilizing the reconstructed oocyte;

(vi) transferring the reconstructed zygote or fertilized oocyte into an oviduct of a recipient female of the same species as the zygote or oocyte; and

(vii) allowing the reconstructed zygote or oocyte to develop to term.

Claim 20 has been amended as follows:

20. (amended) The method of claim 19, wherein the transgene codes for a protein selected from the group consisting of human growth hormone, interferon, β-casein, α-1 antitrypsin, antithrombin III, collagen, factor VIII, factor IX, factor X, fibrinogen,hyaluronic acid, insulin, lactoferrin, protein C, erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), tissue-type plasminogen activator (tPA), feed additive enzymes, somatotropin, ~~and~~ chymotrypsin, monoclonal antibodies, and polyclonal antibodies.

Claim 21 has been amended as follows:

21. (amended twice) A method of producing a protein, comprising:

(i) producing a transgenic ~~avian~~ chicken according to the method of claim 19 wherein the transgene encodes an exogenous protein, said protein being deposited ~~in~~ into ~~the white of~~ the developing eggs of said ~~avian~~ chicken;

(ii) harvesting hard shell eggs of said chicken; and

(iii) isolating the exogenous protein from said eggs.

Claim 24 has been amended as follows:

24. (amended) A method of claim ~~19~~ 14 wherein the ~~avian~~ cloned chicken is a knock-out or knock-in ~~avian~~ chicken.

Claim 25 has been amended as follows:

25. (amended) An intact hard shell egg produced by the method of claim 21 containing ~~less than the normal complement of endogenous proteins found in the egg~~ exogenous protein.

Claim 26 has been amended as follows:

26. (amended) A ~~reconstituted avian~~ reconstructed chicken embryo ~~prepared by transferring the nucleus of a donor cell into a suitable recipient cell~~ comprising a nucleus from a first donor cell in the cytoplast of a second suitable recipient cell.

Claim 27 has been canceled.

Claim 28 has been amended as follows:

28. (amended) A method of producing a cloned ~~avian~~ chicken comprising:

(i) producing a reconstructed zygote by the ~~process~~ method of claim 1;

(ii) transferring the reconstructed zygote into an oviduct of a recipient female of the same species as the zygote; and

(iii) allowing the reconstructed zygote to develop to term.

Claim 29 has been canceled.

Claim 30 has been amended as follows:

30. (amended) A method of producing a cloned ~~avian~~ chicken comprising:

(i) producing a reconstructed oocyte by the ~~process~~ method of claim 1;

(ii) fertilizing the reconstructed oocyte to produce a reconstructed zygote;

(ii) transferring the reconstructed zygote into an oviduct of a recipient female of the same species as the zygote; and

(iii) allowing the reconstructed zygote to develop to term.

Claim 31 has been canceled.